EFFECT OF POLYETHYLENE GLYCOL CONCENTRATIONS ON GROWTH AND PROLINE CONTENT OF *TACCA LEONTOPETALOIDES* SHOOTS CULTURED *IN VITRO*

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Abstract

Tacca leontopetaloides (called taka) is a tuberous plant producing high carbohydrate content useful as functional food. This plant grows in some limited coastal area in Indonesia. Tissue culture of this plant has been done for micropropagation and for *in vitro* conservations. Genetic improvement is important to produce genotypes which are able to grow in a marginal land such as in a drought condition. The aim of this research was to investigate the effect polyethylene glycol (PEG) concentrations added to culture medium on growth and proline content of taka shoots cultured*in vitro*. Shoots of taka were treated with 2.5-15% PEG. After 6 weeks of treatments, growth was evaluated by recording height of shoots, number of shoots, number of leaves, number of roots and fresh weight. Proline content was also determined at the same time. The results showed that growth of taka decreased along with increase in PEG concentrations. In contrary, proline content in taka explants increased along with increase in PEG concentrations. Taka produced few roots on the medium added with high level of PEG (7.5-15%).

***Keywords**:Tacca leontopetaloides, polyethylene glycol (PEG), proline content, growth,in vitro*

1. Introduction

Water stress is a major abiotic factor that limits crop productivity, restrict plant distribution, growth, and yield [1]. The insufficient water status in plant leads to water deficit or drought. Drought or water deficit is not only caused by lack of water, but also because of environmental condition such as low temperature and salinity, thus plants share many molecular compounds in response to these stresses[2]. There are two principal strategies in response to water stress i.e. synthesis of protective molecule during water stress to prevent damage and repair-based mechanism during rehydration to neutralize damage[2].

The plant in osmotic stress (salinity and drought) has been reported to synthesize and hyper-accumulate protective molecules such as soluble carbohydrate, amino acids, betaines and proline [3]. Proline is a proteinogenic amino acid with an exceptional conformational rigidity, and essential for primary metabolism. Proline accumulation was reported to occur during conditions of drought, high salinity, high light and UV radiation, heavy metals, oxidative stress and in response to biotic stresses [4]. Proline is often considered to act as a compatible solute involved in osmotic adjustment at the plant cell level, although the precise role of this accumulation in osmotic adjustment is still debated, proline is accumulated in cytoplasm without having a detrimental effect on cytosolic enzyme activities [3].

Plant responses to abiotic stresses are complex phenomenon with specific characteristic among various species, thus understanding mechanism involved in stress response of plants is necessary [5]. *In vitro* culture is a tool that frequently used for studying of stress tolerance mechanism because its advantages in controlling the physical environment, nutrition, and homogeneity[1]. Various studies have been conducted by using polyethylene glycol (PEG) in tissue culture as osmotic agent such as in castor bean[1], rice [6], wheat [7], *Atriplex*[8], soybean [9],pigeonpea[10], and tomato[11]. PEG is a neutral polymer and is available in different molecular mass[12] and it has been used for several research on plant water stress [6–17]. PEG with high molecular weight is often used for inducing water stress in *in vitro* plant culture research because it is chemically inert, does not enter the cell, does not cause toxicity, thus it is satisfactorily simulating the drought effects[18].The molecular weight of PEG used for the study of water stress were various i.e. PEG-4000 [6,12], PEG-6000 [11,13,17] and PEG-10000[8,9,19].

Polynesian arrowroot (*Tacca leontopetaloides (L.*) Kuntze Syn. *T. pinnatifida* Forst, *T. involucrata*Schum and Thonn.) is a species of flowering plant and recently were grouped into family D*ioscoreaceae*[20,21]. Physicochemical analysisfrom *T. leontopetaloides*starch revealed that the starchis similar to those of potato and maize starch, and*Tacca* starch was relatively more resistant to compression. This particular attribute of *tacca*starch could make it to be used for pharmaceutical excipient comparable to maize starch in tablet formulation[22].*Tacca*was often found in thedappled shade behind sandy beaches and therefore this plant was suspected to be tolerant against osmotic stresses such as drought and salinity. The tissue culture of *Tacca leontopetaloides* has been done[23] and thus the aim of this study was to investigate the effect of drought induced by PEG on growth of *in vitro* culture and proline content of*T. leontopetaloides*.

2. Methods

Plant culture materials and PEG treatment

The corms of *T. leontopetaloides* used in this experiment were came from two-month-olds shoots of *T. leontopetaloides* cultured*in vitro*. The cultures were grown in MS medium [24] supplemented with 0.5 ppm Benzyl Amino Purine (BAP)and 30 g/L sucrose. The medium was solidified with 8 g/L agar and adjusted to pH 5.8 with 1N KOH. The medium was autoclaved at 121 ^{0}C and 103 kPa for 15 min. The corms were planted on MS solid medium supplemented with various concentrations of PEG (MW 4000) at 0; 2.5; 5; 7.5; 10; 12.5 and 15%, respectively. The cultures were incubated at 25 ± 2 ^{0}C under continuous light provided by cool white fluorescent tube with 1000-1400 lux light intensity.

Growth parameters

The height of shoots, number of shoots, and number of roots per explant were recorded 6 weeks after culture. The shoot fresh weight per explant was also recorded after 6 weeks of culture.

All data were analyzed by variance analysis (ANOVA), followed by Duncan’s Multiple Range Test (DMRT) at 5% probability from themean comparison.

Determination of proline concentration

After six weeks of culture, whole parts of explants were harvested for proline analysis. Purified proline was used as standard for proline quantification. The proline assay was determined as described by Bates et al.[25]. The acid-ninhydrin reagent was prepared by warming 1.25 g ninhydrin in 30 mL of glacial acetic acid and 20 mL of 6 M phosphoric acid, agitated and dissolved. Approximately 0.5 g of plant material was homogenized in 10 mL of 3% sulfosalicylic acid and filtered by Whatman no.2 filter paper. Two mL of filtrate was reacted with 2 mL acid-ninhydrin and 2 mL of glacial acetic acid in a test tube for 1 h at 100^{o}C, and the reaction was terminated in ice bath. The reaction mixture was extracted with 4 mL toluene, mixed with stirrer for 15-20 sec. The chromophore containing toluene was aspirated from the aqueous phase, warmed to a room temperature and read the absorbance at 520 nm with toluene as a blank. The proline concentration was determined based on a standard curve and calculated on a fresh weight basis as follows: [(µg proline/mL x mL toluene)/115.5 µg/µmole] / [(g sample)/5] = µmoles proline / g of fresh weight material.

3. Results and Discussion

After six weeks in culture (Figure 1), thegrowth of *T. leontopetaloides*shoot cultu resreduced along with the increase of PEG concentrations. The growth of *T. leontopetaloides* culture were qualitatively observed to be reduced in addition of PEG from 7.5 up to 15%.

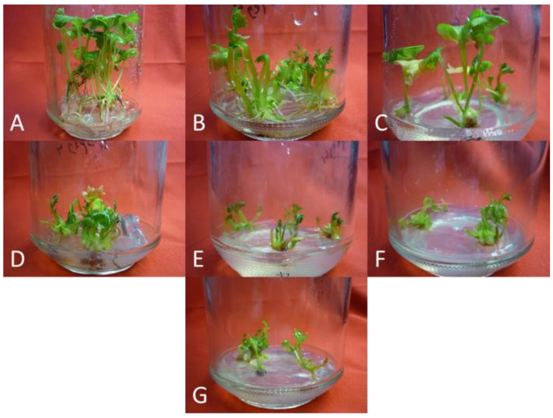

Figure 1. *T. leontopetaloides* culture after six weeks in the treatment medium: (A) MS medium (control); (B) 2.5% PEG; (C) 5% PEG; (D) 7.5% PEG; (E) 10% PEG; (F) 12.5% PEG; (G) 15% PEG.

The addition of PEG into the medium induced an inhibiting effect on growth of *T. leontopetaloides*culture. From data shown inFigure 2, the shoot height decreases along with the increase of PEG concentration. As data showedin Figure 2, theaddition of 2.5 and 5% PEG had already decreased the shoot height of *T. leontopetaloides* culture significantly. The lowest shoot height wasachieved at culture treated with 15% PEG treatment at 1.088 ± 0.040 cm whilst the highest shoot height was recorded from control treatment at 2.417 ± 0.170 cm.

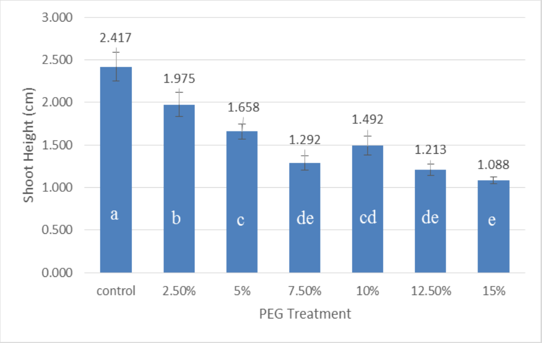

Figure 2. The effect of PEG with various concentration on shoot height of *T. leontopetaloides* in the sixth week after subculture. Bar with different letter is significantly different (*P=0.05*) according to DMRT.

The number of shoots increased with the addition of 2.5 to 5% of PEG treatment (Figure 3). As data shown in Figure 3, PEG addition from 10 to 15% inhibitedformation of new shoots. The highest number of shoots were recorded from 2.5% PEG treatment at 1.417 ± 0.416 per explant and significantly different from the control treatment.The lowest number of shoots were recorded from 10, 12.5, and 15% PEG treatment at 1.000 ± 0.000. This indicated that with those PEG concentration treatments there are no new shoot formed.

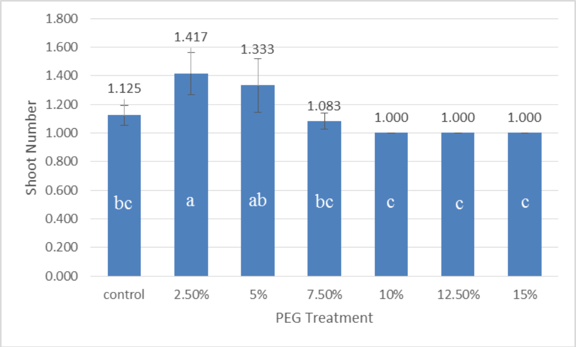

Figure 3. The effect of PEG with various concentration on number of shoot of *T. leontopetaloides*in the sixth week after subculture. Bar with different letter is significantly different (*P=0.05*) according to DMRT.

The highest number of leaves was recorded from 2.5% PEG treatment at 3.500 ±0.565, however, it is not significantly different from the control treatment (Figure 4). Similar to number of shoots parameter (Figure 3), number of leaves increased with addition of 2.5 up to 5% PEG (Figure 4). The growth inhibition effect on number of leaves was strongly seenat 7.5 up to 15% PEG treatments. The lowers number of leaves were from 12.5% PEG treatment at 0.750 ± 0.202.

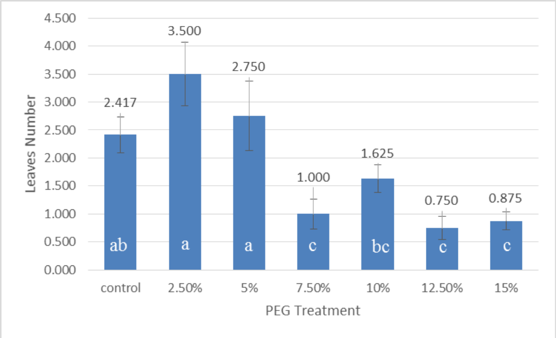

Figure 4. The effect of PEG with various concentration on number of leaves of *T. leontopetaloides*in the sixth week after subculture. Bar with different letter is significantly different (*P=0.05*) according to DMRT.

Similar data trend were recorded on number of roots parameter that number of roots increasedfrom 2.5 to 5% PEG treatment compared to the control. The highest number of roots per explant were obtainedat 5% PEG treatment at 1.083 ± 0.275 and significantly different compared to the control (Figure 5). PEG treatment from 7.5 up 15% inhibited the root formation significantly. The roots were not formed in the 12.5% PEG treatment as data shown inFigure 5.

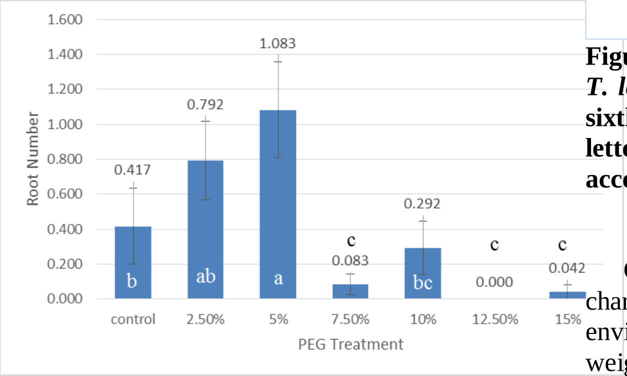

Figure 5. The effect of PEG with various concentration on number of roots of *T. leontopetaloides*in the sixth week after subculture. Bar with different letter is significantly different (*P=0.05*) according to DMRT.

Figure 6 shows that fresh weight (FW) of *T. leontopetaloides*explantdecreased along with the increase of PEG concentrations. The highest fresh weight was obtained from control treatment (0.859 ± 0.121 g) whilst the lowest fresh weight was obtained from 15% PEG treatment (0.245 ± 0.030 g). In contrary, proline content increased along with the increase of PEG concentrations. The highest proline content was obtained from 15% PEG treatment (1.675 µg/g FW), whilst the lowest proline content was obtained from control treatment (6.875 µg/g FW).

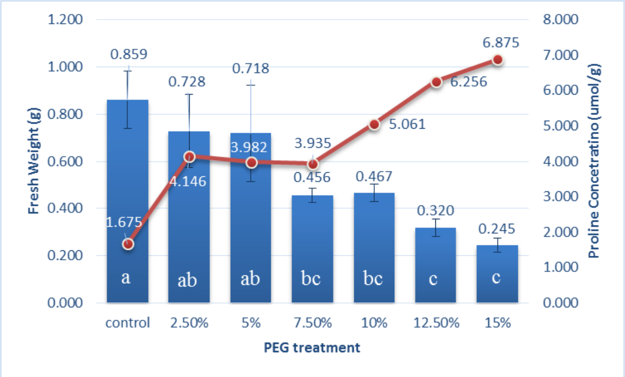

Figure 6. The effect of PEG on fresh weight of *T. leontopetaloides*and its proline content in the sixth week after subculture. Bar with different letter is significantly different (*P=0.05*) according to DMRT.

Growth analysis is a fundamental characterization toassess' plant's responses to environmental stress [26]. The high-molecular-weight PEG has been used to study *in vitro* induction water deficit because it reduces the osmotic potential of the medium [18]. In our present studyosmotic stress induced by PEG from 7.5 to 15% resulted in decrease of growth significantly as data shown on Figures 1-5. In lower level of PEG (at 2.5 – 5% PEG) the cultures were able to maintain growth as shown in Figure 3-5. Number of shoots and number of leaves were slightly higher compared to the control, although it was not significantly different. Interestingly the root numbers increased significantly in the present of 5% PEG. This result was similar to research finding in germination of cotton cultivar [13]that had radicle length increased in the present of low level of PEG.

The fresh weight of *T. leontopetaloides*culture tends to decrease along with the increase of PEG concentrations. Even though the decrease of FW in 2.5 and 5% PEG are not significantly different compared to the control. The decrease of FW was significantly different compare to the control on 7.5 up 15% PEG. This indicates that *T. leontopetaloides* culture has the ability to sustain their water content to mild stress, whereas this ability lost under severe stress treatment. Similar result was reported in pigeonpea[10]where the plant was able to maintain growth under PEG mild stress. It has been shown that proline has a key role in stabilizing cellular protein and membranes in presence of high concentration of osmoticum [27]. In our previous work, proline concentrations increased in the presence of stress osmoticum

(NaCl)[28,29]. In our present work proline concentrations began to increase at 2.5% and at higher than 15% PEG.Similar result was also reported in rice calli [17], pegionpea (*Cajanus cajan*)[10]. The accumulation of proline during drought is related to its basic chemical properties whereas proline is the most water soluble amino acids and exists much of the time in zwitterionic state having weak negative and positive charges at the carboxylic acid and nitrogen groups, respectively [30]. In our present work, it seems that proline was effective in maintaining growth from 2.5 to 5% PEG as shown inFigures 3, 4 and 5. This was also because of characteristic of proline whereas proline capable to maintain a hydration sphere around the biopolymers and maintain their native state, thereby regulating growth under drought and salinity stresses [31].Thus, it can be concluded that addition of PEG into the medium clearly indicated water stress effect on *T. leontopetaloides* culture. Even though*T. leontopetaloides*culture could maintain growth from 2.5 to 5% PEG, *T. leontopetaloides* culture was not able to tolerate higher PEG concentrations which were from7.5 to 15%.

4. Conclusion

Shoot height and Fresh weight of *T. leontopetaloides* culture decreased along with the increase of PEG concentration, whereas the number of shoots, number of leaves and number of roots slightly increased in the presence of 2.5 up to 5% PEG then decreased at high level of PEG (from7.5 to 15%). Proline level increased along with the increase of PEG concentrations.

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